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Genetic Basis for Using 'Tradescantia' Clone 4430 as an Environmental Monitor of Mutagens

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16. ABSTRACT

The Tradescantia stamen hair system developed by the late Dr. Arnold H. Sparrow has been used in a wide variety of radiobiological studies[1,3,4,8,11,14], and more recently as an environmental monitor for assessing the potential genetic hazards of various gasesous chemicals of both industrial and natural origin[2,6,9-11,16]. The use of this system as a genetic monitor necessitates a thorough genetic analysis of the marker employed to measure mutagenicity. The phenotypic change in color from blue to pink in either petal or stamen hair cells has been used as a genetic marker under the assumption that the petal and stamen hair cells are heterozygous for blue, and if the dominant allele for blue color mutates or is lost, the recessive allele determines that the daughter cells will be pink. It is the purpose of this communication to present the results of genetic test by conventional breeding methods involving the pink locus in Tradescantia clone 4430, the diploid clone used exclusively in the Mobile Monitoring Vehicle[9] at Brookhaven National Laboratory. Confirmation of a genetic, as opposed to a physiological, orgin of the pink color int he petal and stamen hair cells of Tradescantia clone 4430 is essential to evaluating the validity of this test system.

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Genetic basis for using *Tradescantia* clone 4430 as an environmental monitor of mutagens

M. EMMERLING-THOMPSON AND M. M. NAWROCKY

THE *TRADESCANTIA* stamen hair system developed by the late Dr. Arnold H. Sparrow has been used in a wide variety of radiobiological studies[1,3,4,8,11,14], and more recently as an environmental monitor for assessing the potential genetic hazards of various gaseous chemicals of both industrial and natural origin[2,6,9–11,16]. The use of this system as a genetic monitor necessitates a thorough genetic analysis of the marker employed to measure mutagenicity. The phenotypic change in color from blue to pink in either petal or stamen hair cells has been used as a genetic marker under the assumption that the petal and stamen hair cells are heterozygous for blue, and if the dominant allele for blue color mutates or is lost, the recessive allele determines that the daughter cells will be pink. It is the purpose of this communication to present the results of genetic tests by conventional breeding methods involving the pink locus in *Tradescantia* clone 4430, the diploid clone used exclusively in the Mobile Monitoring Vehicle[9] at Brookhaven National Laboratory. Confirmation of a genetic, as opposed to a physiological, origin of the pink color in the petal and stamen hair cells of *Tradescantia* clone 4430 is essential to evaluating the validity of this test system.

Material and Methods

Since little information has been published on clone 4430, and because it is now widely used it seems important to present all the data that we have collected over a period of years. Table I lists the origin, cytological data, spontaneous and induced mutation rates, and breeding information on clone 4430.

In the present study, blue-flowered clone 4430 was test-crossed to its pink-flowered parental clone 2441 in order to confirm its heterozygosity and to determine whether the genes governing pink and blue color are inherited sexually as independent entities (Table II). In addition, second generation or simulated F_2 data were obtained by crossing clone 4430 with heterozygous sibling plants from the original interspecific cross (Table III). Also, preliminary experiments were conducted to determine the number of white or color-determining loci present in clone 4430 (Table IV).

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Several hundred progeny were examined from these three different types of crosses, and each is dealt with separately. Details of the pollinating technique, handling of the progeny, and the determination of flower color have been described elsewhere[15].

Table I. Summary of miscellaneous information pertaining to *Tradescantia* clone 4430

A. Origin of clone 4430[11]
   blue-flowered *T. hirsutiflora** (2461C) × pink-flowered *T. subacaulis*† (2441)
B. Somatic chromosome number
   T. hirsutiflora $2n = 12$ (Figure 1*A*)
   T. subacaulis $2n = 12$ (Figure 1*B*)
   clone 4430 $2n = 12$ (Figure 1*C* and *D*)
C. Nuclear volume[13]
   T. hirsutiflora 758.4 μ^3
   T. subacaulis 756.9 μ^3
   clone 4430 801.1 μ^3
D. Interphase chromosome volume[13]
   T. hirsutiflora 63.2 μ^3
   T. subacaulis 63.1 μ^3
   clone 4430 66.8 μ^3
E. Percentage of spontaneous pollen abortion
   T. hirsutiflora 9.8
   T. subacaulis 6.2
   clone 4430 10.0
F. Classification of flower color according to R.H.S.‡[7]
   T. hirsutiflora 93C
   T. subacaulis 73B
   clone 4430 94C
G. Spontaneous mutation rate per hair[12]
   clone 4430—2.61 ± 0.9 to $5.21 \pm 0.20 \times 10^{-3}$
H. Radiation-induced mutation rate per hair—100 rad X-rays[11]
   clone 4430—$126 \pm 5.0 \times 10^{-3}$
I. Chemically-induced mutation rate per hair—60 ppm DBE[11]
   clone 4430—$25.3 \pm 3.6 \times 10^{-3}$
J. Chemically-induced mutation rate per hair—50 ppm EMS[11]
   clone 4430—$46.0 \pm 5.3 \times 10^{-3}$
K. Ability to set seed by selfing or crossing
   T. hirsutiflora—self sterile
   —cross with *T. subacaulis* and clone 4430
   T. subacaulis—self sterile
   —cross with *T. hirsutiflora* and clone 4430
   clone 4430—self sterile
   —cross with *T. hirsutiflora* and *T. subacaulis*

* Collected by C. A. Brown near Kinder, LA
† Collected by D. B. Horne near Huntsville, TX
‡ According to Royal Horticultural Society Colour Chart[7]

Results and Discussion

Testcrosses of clone 4430. Table II summarizes the results from reciprocal testcrosses of hybrid clone 4430 with its original pink-flowered parental clone 2441 (*T. subacaulis*). Clone 4430 was used as a male parent in nine crosses, and as a female parent in eight crosses. All 17 plants of cone 4430 were obtained by vegetative propagation from the original 4430 hybrid plant. Self-contamination was eliminated since both clones

Table II. Phenotypes and segregation ratios from testcrosses of *Tradescantia* clone 4430

Parent no.	No. blue-flowered plants	No. pink-flowered plants	Total no. examined	Ratio tested	χ_1^2	*P*
A. Male testcross of clone 4430						
2441 × 4430-1	8	13	21			
-2	7	2	9			
-3	12	10	22			
-4	13	14	27			
-5	10	12	22			
-6	10	16	26			
-7	7	9	16			
-8	10	4	14			
-9	2	4	6			
Total	79	84	163	1:1	0.15	>0.05
Total no. pollinations—531						
Max. no. seed exp.—3186						
Actual no. seed produced—1126						
Total no. mature plants—163						
B. Female testcross of clone 4430						
4430-1 × 2441	87	102	189			
-2	84	76	160			
-3	19	23	42			
-4	52	53	105			
-5	18	21	39			
-6	18	33	51	1:1	4.41	<0.05
-7	37	40	77			
-8	19	26	45			
Total	334	374	708	1:1	2.26	>0.05
Total (without #6)	316	341	657	1:1	0.95	>0.05
Total no. pollinations—846						
Max. no. seed exp.—5076						
Actual no. seed produced—1177						
Total no. mature plants—708						

Table III. Phenotypes and segregation ratios from sibling crosses of *Tradescantia* clone 4430

Parent no.	No. blue-flowered plants	No. pink-flowered plants	Total no. examined	Ratio tested	χ_1^2	*P*
With sibling HH-1						
HH-1 × 4430	11	5	16			
4430-1 × HH-1	31	7	38			
4430-2 × HH-1	42	14	56			
4430-3 × HH-1	27	6	33			
Total	111	32	143	3:1	0.52	>0.05
Total no. pollinations—457						
Max. no. seed exp.—2742						
Actual no. seed produced—538						
Total no. mature plants—143						
With sibling HH-25						
HH-25 × 4430	8	5	13			
4430-1 × HH-25	28	6	34			
4430-2 × HH-25	30	6	36			
4430-3 × HH-25	19	4	23			
Total	85	21	106	3:1	1.52	>0.05
Total no. pollinations—316						
Max. no. seed exp.—1896						
Actual no. seed produced—458						
Total no. mature plants—106						

FIGURE 1—Root-tip metaphase chromosomes. *A—T. hirsutiflora* with 3 or possibly 4 satellited chromosomes; *B—T. subacaulis* with 4 satellited chromosomes; *C—Tradescantia* clone 4430 with 3 or 4 satellited chromosomes; and *D*—microspore metaphase of *Tradescantia* clone 4430 showing two satellited chromosomes (×1550).

4430 and 2441 are completely self sterile. In addition, these crosses were made by hand in isolated growth chambers.

At present, a total of 871 plants have been examined from reciprocal testcrosses of clone 4430. Of the 163 progeny obtained in nine crosses using clone 4430 as the male parent (Table II A), 79 had blue flowers and 84 had pink flowers. The numbers observed agree closely with an expected ratio of 1:1 ($\chi^2_1 = 0.15$, $P > 0.05$). Of the 708 progeny obtained in eight crosses using clone 4430 as the female parent (Table II B), 334 were blue-flowered and 374 were pink-flowered plants. Again, these results are in reasonable agreement with 1:1 ratio ($\chi^2_1 = 2.26$, $P > 0.05$). If cross 6 of the female test, which gives the most anomalous ratio, is excluded from the totals, the observed numbers agree even more closely with the expected ratio of 1:1 ($\chi^2_1 = 0.95$, $P > 0.05$). The deficiency of blue-flowered progeny in cross 6 could, however, be attributed to sampling error from a 1:1 ratio.

The data from reciprocal testcrosses are consistent with the interpretation that pink flower pigmentation in clone 4430 is determined by a single pair of alleles where blue floral color (*B*) is dominant to pink (*b*). It may be concluded from the observed 1:1 testcross ratios that clone 4430 is heterozygous (*Bb*) for pink flower color. This was not the case for *Tradescantia* clone 02 where a deficiency of pink-flowered progeny was observed by Mericle and Mericle[5] and Sparrow and Nawrocky (unpublished data).

It is possible to speculate from these data that pink petal and stamen hair cell color is controlled by a single gene, or by two very closely linked genes. The 413 plants with blue-colored flowers always had blue stamen hair cells and the 458 pink-

flowered plants invaribly had pink stamen hair cells (Table II). The blue-flowered progeny were not scored exhaustively for single mutated pink stamen hair cells, although control levels were expected, nor were the pink-flowered plants examined for an occassional blue stamen hair cell. The later event is so rare in clone 4430 as to be virtually undocumented in our laboratory.

Sibling crosses with clone 4430

Table III shows the results from crossing clone 4430 with two heterozygous sibling F_1 plants, HH-1 and HH-25. These sibling plants were selected from the progeny of the original interspecific cross of *T. hirsutiflora* × *T. subacaulis* that included clone 4430. These simulated or controlled F_2 data were necessarily obtained by this breeding method, rather than by selfing clone 4430, since the latter is completely self-sterile.

Progeny from the cross of clone 4430 with sibling HH-1 consisted of 111 blue- and 32 pink-flowered plants, agreeing closely to a hypothetical 3:1 ratio ($\chi^2_1 = 0.52$, $P > 0.05$). Similarly, the cross of clone 4430 with sibling plant HH-25 produced 106 plants of which 85 were blue and 21 were pink. This ratio also was not significantly different from 3:1 ($\chi^2_1 = 1.52$, $P > 0.05$).

These results corroborate the testcross data and are consistent with the hypothesis that pink flower color segregates in clone 4430 as a single factor recessive.

Crosses of clone 4430 with white-flowered tester stocks

Additional genetic information is available with regard to clone 4430 from crosses with white-flowered tester stocks. Past studies[15] have shown that blue petal color in *T. subacaulis* and *T. humilis* results from the interaction of two pairs of dominant independent genes, and that white petal color arises when either one or both genes is homozygous recessive. Consequently, crosses between blue-flowered clone 4430 and white-flowered *T. subacaulis* (*ccRR*) or white-flowered *T. humilis* (*CCrr*) would be expected to produce 1 blue: 1 white if clone 4430 is heterozygous for either one or both of the two white loci.

Table IV shows the results of crossing clone 4430 with white-flowered *T. subacaulis*. Germination and sterility problems are apparent from this table. Among 244 seeds planted, only 81 plants survived to maturity, and all were blue-flowered. These results suggest that clone 4430 is homozygous dominant for the white locus of *T. subacaulis*.

Table IV also shows the results from crossing clone 4430 with white-flowered *T. humilis*. Again, sterility and germination problems were encountered. In the cross where clone 4430 was used as the female parent, no seed was produced from 255 pollinations. The reciprocal cross employing white-flowered *T. humilis* as the female parent produced 93 seeds, but only 10 plants survived to produce flowers, and all were blue-flowered. Although this sample is small and pollinations will be repeated, the results are significantly different from a 1:1 ratio ($\chi^2_1 = 10.0$, $P < 0.01$) and suggest that clone 4430 is also homozygous dominant for the white locus of *T. humilis*.

Summary

Inheritance of pink pigmentation in petal and stamen hair cells of *Tradescantia* clone 4430 was studied in reciprocal testcrosses of clone 4430 with its pink-flowered parental clone 2441 and in crosses with sibling F_1 plants. The results from these crosses are consistent with the hypothesis that pink pigmentation is determined by a pair of alleles at a single locus, and that blue (*B*) is dominant to pink (*b*).

Preliminary evidence from crosses of clone 4430 with white-flowered *T. humilis* and with white-flowered *T. subacaulis* further suggests that clone 4430 is homozygous dominant for both the *R* and *C* white loci necessary to produce any pigmentation.

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Table IV. Data from crosses of clone 4430 with white-flowered tester stocks

Parent no.	No. pollinations	Max. no. seed exp.	Actual no. seed prod.	No. blue-flowered plants	No. white-flowered plants
A. Test with white-flowered *T. subacaulis*					
4430 × 2451	71	426	113	51	0
2451 × 4430	59	354	131	30	0
Total	130	780	244	81	0
B. Test with white-flowered *T. humilis*					
4430 × 2868					
Exp. #1	56	336	0	0	0
Exp. #2	106	636	0	0	0
Exp. #3	93	558	0	0	0
2868 × 4430	34	204	93	10	0
Total	289	1734	93	10	0

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